4/5/88

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
	:	
ETSUO OSHIMA, et al.	)	
	:	Group Art Unit: 129
Serial No.: 020,900	)	
	:	Examiner:
Filed: March 2, 1987	)	Richard L.Raymond
	:	
For: DIBENZ[b, e]OXEPIN DERIVATIVE	)	

DECLARATION

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

I, KENJI OHMORI, of 2-14-3, Fuyodai, Mishima-shi, Shizuoka-ken, Japan, hereby declare as follows:

I graduated from the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kyoto University in March, 1970. I have been employed since April, 1970 by Kyowa Hakko Kogyo Co., Ltd. where I am engaged in the research and development of antiallergic agents. I studied the evaluation of antiallergic agents at the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kyushu University from April, 1975 to March, 1976 at the direction of Kyowa Hakko Kogyo.

I am one of the inventors of the above-identified application and well acquainted with the prosecution thereof.

I have conducted the following experiments comparing, with respect to antiallergic activity (Anti PCA

activity) and side effect (M_1 binding affinity), between compounds of the present invention and certain of the compounds disclosed in the references cited by the Examiner.

M_1 (Muscarinic acetylcholine receptor) binding affinity shows the index of the side effect such as suppression of salivary secretion and mydriasis, caused by anticholinergic effect.

The smaller Ki-value shows closer affinity to the receptor and thus, the side effect is liable to be caused.

Experiment

1. Anti PCA (passive cutaneous anaphlaxis) activities:

The experiment was conducted according to the same method described in this application, P37 ℓ9 - P38 ℓ28.

The results are shown in Table I - III.

2. M_1 binding affinities:

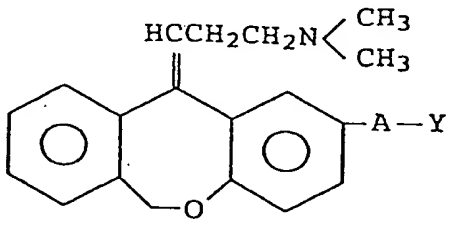
The binding assay was carried out according to the method [J. Neurochem., 32, 421 (1979)] with minor modification. The striatum of rat was homogenized in 10 volumes of distilled water with Potter-Elvehjem homogenizer. This homogenate preparation was diluted by 200 volumes of the wet tissue weight with 50 mM Na-K phosphate buffer solution (pH 7.4). 50μℓ of ^{3}H-quinuclidinyl benzilate solution (final concentration 1.26 nM) and the test compound solution (10% ethanol, 50 μℓ) were added to 1 mℓ of homogenate (corresponding to 5 mg wet tissue) and incubated at 37°C for 60 min. Nonspecific binding was determined by addition of unlabeled dexetimide (10% ethanol solution, final concentration 1 μM). The assay was terminated by rapid filtration under reduced pressure over Whatman GF/B filter. The filters were rinsed 3 times with 5 mℓ of ice-cold 50 mM Na-K phosphate buffer (pH 7.4), transferred to counting vials

containing 7 mℓ of scintillator (Scintisole EX-H, Wako) and counted by liquid scintillation spectrometry (Packard Tri-carb 330).

Log IC_{50}-values (IC_{50}: concentration producing 50% inhibition of the specific binding of the ^3H-ligand) were derived from plots of the percentage inhibition of specific ^3H-ligand binding versus the log concentration of the compounds. Ki-values were calculated according to Cheng and Prusoff [Biochem. Pharmac., 22, 3099 (1973)], using mean IC_{50}-values from 3 independent determinations: $Ki=IC_{50}/[1+C/K_D]$, Ki and K_D equilibrium dissociation constants of the test compound (inhibitor) and the ^3H-ligand respectively, C: concentration of the ^3H-ligand.

The results are shown in Tables I - III.

Table I

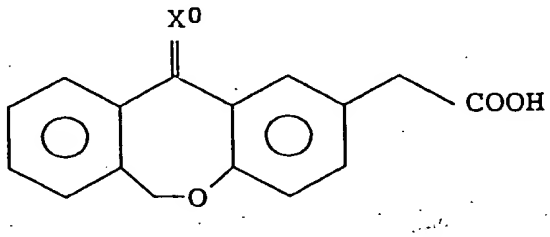


Compound	-A-Y		Anti PCA MED (mg/kg, PO)	M_1 Binding Ki (μM)
3	-COOH	(Z)	0.1	-**
3	"	(E)	0.1	-
20	$-CH_2COOH$	(Z)	0.01	-
20	"	(E)	0.1	-
28	$-CH_2CH_2COOH$	(Z)	0.1	-
28	"	(E)	0.01	-
60	-CH=CHCOOH	(Z)	0.1	-
A* (Doxepin)	H	(Z)	1.0	0.04
B*	$-CH_3$	(Z)	1.0	0.26
B*	"	(Z/E=2/1)	1.0	0.28

* Compound A is the compound prepared in Example VI, USP 3,420,851 and Compound B is the compound prepared in Example X, the 11th compound of the table on column 16.

**"-" means the percentage inhibition is less than 50% even at the concentration of 100 μM of the test compound.

Table II



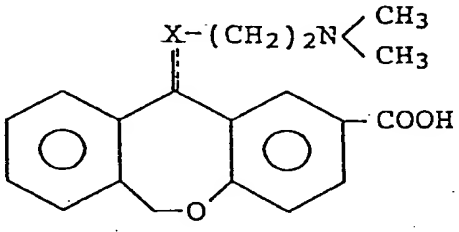
Compound	=X0	Anti PCA MED (mg/kg,PO)	M_1 Binding Ki (μM)
20	$=CHCH_2CH_2N(CH_3)_2$ (Z)	0.01	-***
20	" (E)	0.1	-
22	$=CHCH_2CH_2CH_2N(CH_3)_2$ (Z)	0.1	-
26	$=CHCH_2N$ (piperazine) NCH_3 (E/Z=12/88)	1.0	-
C* (Isoxepac)	=O	>100	-
D**	$=CH_2$	>100	-

* Compound C is Compound No. 2, Table I, page 942 J. Med. Chem., 19, 941 (1976) and Compound No. 7, Table I, page 1500 ibid, 20, 66 (1977); this application (P46 Reference example 2)

** Compound D is described in this application (P47 reference example 8)

*** "-" means the percentage inhibition is less than 50% even at the concentration of 100 μM of the test compound.

Table III



Compound	=====X		Anti PCA MED (mg/kg,PO)	M_1 Binding Ki (μM)
3	=CH-	(Z)	0.1	-**
3	=CH-	(E)	0.1	-
E*	-NH-		1.0	-

* Compound E is obtained by hydrolyzing compound 13 according to the method of Example 16 in USP 4,596,804.

** "-" means the percentage inhibition is less than 50% even at the concentration of 100 μM of the test compound.

3. Conclusion:

It is concluded that the test compounds of the present invention exhibit excellent antiallergic activity and are almost free from affinity to the receptor, and that is to say, they have less side effect.

The undersigned declarant declares further that all statements made herein of his knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonments, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Executed this 29th day of February , 1988.

Kenji Ohmori

KENJI OHMORI